

Review

Genomics and Ecology of Novel N₂O-Reducing MicroorganismsSara Hallin,^{1,*} Laurent Philippot,² Frank E. Löffler,^{3,4} Robert A. Sanford,⁵ and Christopher M. Jones¹

Microorganisms with the capacity to reduce the greenhouse gas nitrous oxide (N₂O) to harmless dinitrogen gas are receiving increased attention due to increasing N₂O emissions (and our need to mitigate climate change) and to recent discoveries of novel N₂O-reducing bacteria and archaea. The diversity of denitrifying and nondenitrifying microorganisms with capacity for N₂O reduction was recently shown to be greater than previously expected. A formerly overlooked group (clade II) in the environment include a large fraction of nondenitrifying N₂O reducers, which could be N₂O sinks without major contribution to N₂O formation. We review the recent advances about fundamental understanding of the genomics, physiology, and ecology of N₂O reducers and the importance of these findings for curbing N₂O emissions.

Microbial Sources and Sinks of Nitrous Oxide: Introducing the Nitrous Oxide Reducers

Inorganic nitrogen compounds are primarily transformed through reduction–oxidation reactions mediated by microbial communities in the biogeochemical nitrogen cycle (Figure 1). Their activity regulates the retention or loss of nitrogen from ecosystems, and determines in which form nitrogen is lost. Nitrous oxide is produced in several nitrogen turnover processes (Box 1). It is an important greenhouse gas with a warming potential nearly 300 times that of CO₂ over 100 years, and the major cause of stratospheric ozone depletion [1,2]. Terrestrial ecosystems account for the majority of planetary N₂O emissions, while aquatic ecosystems contribute one third [1], of which the anthropogenic origins can be assigned to agricultural soils [3,4], and agricultural run-off and animal waste [5], respectively. These emissions are expected to increase due to increased use of fertilizers and mineralization of soil organic matter following land use change [6,7].

Although multiple sources of N₂O exist, there is only one known sink for N₂O in the biosphere – the microbial reduction of N₂O to N₂, catalyzed by the N₂O reductase (NosZ). This enzyme has traditionally been assigned to bacteria and archaea performing complete **denitrification** (see Glossary): a pathway ending with N₂O reduction to N₂ [8,9]. A few nondenitrifying bacteria, such as *Wolinella succinogenes* and *Campylobacter fetus*, were reported early on for their capacity to grow with N₂O as the sole electron acceptor [10,11]. Due to the increasing number of sequenced microbial **genomes** from a range of taxa, the diversity of both denitrifying and nondenitrifying microorganisms with the genetic capacity for N₂O reduction was recently shown to be greater than previously expected [12–14]. In contrast to denitrifying microorganisms, nondenitrifying N₂O-reducing bacteria and archaea have the potential to be true N₂O sinks without contributing to N₂O production (Box 1, Figure 1). Agricultural production and

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N₂O is produced in several microbial processes, but N₂O reduction is limited to a single process catalyzed by the N₂O reductase present among taxonomically diverse bacteria and archaea. This enzyme is used for energy conservation through anaerobic respiration, detoxification, or removal of excess electrons.

The N₂O reductase has evolved into two lineages, clade I and the recently described clade II. Surveys indicate that clade II N₂O reductase (NosZ) is abundant in many biomes.

Genome analyses and culture-based studies suggest that the physiology of N₂O reduction differs between the clades. Evidence from community ecology supports niche differentiation of organisms with either type of N₂O reductase, and within the two clades.

Identifying factors that drive physiological and ecological responses of N₂O-reducing communities could lead to effective and innovative strategies to curb N₂O emissions.

¹Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant Pathology, 75007 Uppsala, Sweden

²Agroécologie, AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comté, F-21000 Dijon, France

³Center for Environmental Biotechnology, Department of Microbiology, Department of Civil and Environmental Engineering, Department of Biosystems Engineering and Soil Science,

water treatment facilities are focal points for N₂O mitigation efforts, and the capacity for microorganisms to act as N₂O sinks is gaining increased attention in the development of strategies that combat N₂O emissions and mitigate climate change [15–17].

An important discovery was the realization that NosZ protein **phylogeny** has two distinct groups, clade I and the newly described clade II [13,14]. These clades have also been known as typical and atypical NosZ [13], but the authors of this review have agreed on using the terminology clade I and II NosZ. It is known that denitrifiers within clade I reduce N₂O at different rates [18], implying that denitrifier community composition could influence *in situ* net N₂O fluxes. Less is known about clade II types, especially the nondenitrifiers, although genomic and physiological differences are emerging (e.g., [19,20]). We review the recent literature on N₂O reducers and focus on genomic, physiological, and ecological information that would explain **niche** differentiation between and within the two clades of N₂O-reducing bacteria and archaea. We also discuss the importance of these findings for mitigation of N₂O emissions, especially from soils.

Evolution and Genomics of N₂O-Reducing Microorganisms

The ability to reduce N₂O is a taxonomically widespread **trait**. **Homologs** of the *nosZ* gene, which code for the catalytic subunit involved in N₂O reduction, are found in approximately 12% of sequenced microbial genomes, representing 12 archaeal and bacterial phyla (Figure 2) isolated from various environments [19]. Phylogenetic analysis indicates that the diversification of NosZ occurred predominantly through vertical inheritance with some instances of horizontal gene transfer, as the *nosZ* phylogeny is largely congruent with organismal phylogeny at higher taxonomic levels [19,21]. While this suggests that N₂O reduction is a potentially ancient trait, the literature offers conflicting scenarios on whether an N₂O reductase appeared before or after the ‘great oxygenation event (GOE)’ of the late Archean, 2.8–2.5 Gyr before present. Isotopic evidence suggests that the denitrification pathway appeared only shortly after the GOE [22]. Copper, a key cofactor for several enzymes, including the N₂O reductase, was likely not biologically available in the reduced conditions prior to the GOE, although recent isotopic evidence suggests that copper concentrations may have been less limiting than previously assumed [23]. The formation of NO, the precursor to N₂O, is expected to have occurred prior to the GOE [24], and iron- and heme-based NO reductases could have generated N₂O prior to the build-up of oxygen levels. In addition, N₂O could have been formed by **chemodenitrification**, an abiotic reaction of nitrite with iron (II) [25,26]. Thus, N₂O reductase activity could have evolved due to the accumulation of N₂O in parallel with nitrite reduction, but before nitrate reduction became significant.

Despite evidence for vertical inheritance, the distribution of *nosZ* genes across microbial taxonomic groups at a finer scale is patchier, as *nosZ* can be present or absent in the genomes of closely related organisms [19,21,27–30]. The polyphyletic distribution of N₂O reduction as a trait suggests that the acquisition or loss of *nos* genes could be influenced by environmental conditions [27,31]. Gene loss through deletion is a common evolutionary phenomenon in bacteria, where loss of functions may result in an increase in **fitness** [32]. Indeed, organisms capable of complete denitrification may downregulate *nosZ* expression when nitrate is replete, but other resources are limiting [33]. This may explain why nearly 40% of all genomes that possess genes encoding the nitrite reductases associated with denitrification lack *nosZ* [19]. This pattern is not randomly distributed across taxa. For example, *nosZ* has not been detected in fungal genomes and is conspicuously absent from the genomes of Actinobacteria or Acidobacteria [19,34]. These highly abundant and diverse phyla are commonly associated with low resource availability or low pH conditions in terrestrial ecosystems [35–38], suggesting a lower frequency of *nosZ* in such environments.

University of Tennessee, Knoxville, TN 37996, USA

⁴Biosciences Division, and Joint Institute for Biological Sciences (JIBS), Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

⁵University of Illinois, Department of Geology, Champaign, IL, USA

*Correspondence:
Sara.Hallin@slu.se (S. Hallin).

The diversity of the *nosZ* gene can be divided into two distinct groups of N₂O reducers, clade I and II [13,14] (Figure 3). This is based not only on the phylogeny of the NosZ protein, but also on differences between the two clades regarding the *nos* gene cluster (NGC) organization, NosZ **translocation pathway**, and frequencies of co-occurrence with other genes associated with denitrification or respiratory ammonification. Clade I consists almost exclusively of Alpha-, Beta-, and Gammaproteobacteria. The *nosZ* genes from the halophilic Euryarchaeota (*Haloarcula* and *Natronomonas*) are also grouped with clade I based on evolutionary distance, but form a subclade from the Proteobacteria. The signal peptides reveal that clade I NosZ is translocated to the periplasm using the twin-arginine translocation pathway, in which proteins are first folded in the cytoplasm and then transported across the cytoplasmic membrane [39]. The NGCs of organisms with clade I *nosZ* have a *nosZ* gene followed by *DFYL* genes involved in protein assembly, and possibly copper transport [40] (Figure 4). A defining feature is the presence of *nosR*, encoding a membrane bound Fe-S flavoprotein likely involved in electron transport to NosZ. Analysis of the co-occurrence of *nosZ* with other genes in the denitrification pathway indicate that organisms with clade I *nosZ* are more likely to be complete denitrifiers, as 83% of genomes with clade I *nosZ* also possess *nirS* or *nirK* genes, which encode the two different nitrite reductases NirS or NirK, respectively [19] (Figure 3).

A diverse range of archaeal and bacterial phyla possess clade II *nosZ*. In contrast to clade I, 51% of the organisms with clade II *nosZ* appear to be nondenitrifying N₂O reducers [19] (Figure 3). Interestingly, approximately 27% of genomes with clade II *nosZ* in an earlier analysis also harbored the *nrfA* gene encoding the formate-dependent nitrite reductase [13], a hallmark for respiratory ammonification (Figure 1, Box 1). However, *Photobacterium profundum* and *Shewanella loihica* possess clade I NosZ, which demonstrates that respiratory ammonification does not exclusively occur with clade II NosZ. *Shewanella loihica* is able to perform complete denitrification and respiratory ammonification [41], a feature shared by a few other bacteria (e. g., *Opitutus terrae* and some *Bacillus* spp.). In contrast to clade I, nearly all clade II *nosZ*, except those from archaea and thermophilic bacteria, possess signal peptides associated with the Sec translocation pathway that extrudes the nascent polypeptide chain into the periplasm, where folding and insertion of cofactors then occurs [42]. Whether the difference in translocation pathway between the two clades has any functional significance beyond the translocation itself, or is merely coincident with the distribution of taxa across the *nosZ* phylogeny is unclear. However, studies of several clade II organisms, including *W. succinogenes*, *Bacillus azotoformans* and *Solitalea canadensis*, indicate that NosZ may be closely associated with the cytoplasmic membrane [11,43]. This is further supported by the presence of an open reading frame (ORF) immediately downstream of *nosZ*, referred to as *nosB* and predicted to encode a membrane-spanning protein involved in electron transport [44]. The halophilic archaea within clade I are an exception to this pattern, as their NGCs also contain *nosB*. Overall, the genomic signatures indicate obvious differences between organisms with clade I and II *nosZ*, but further biochemical and physiological studies are required to determine how these differences in NGC arrangement and translocation pathway translate into physiological differences that potentially drive niche differentiation within and between each clade.

Physiology of N₂O-Reducing Bacterial Populations

The distribution of *nosZ* genes in bacterial genomes and the multiple sources of N₂O formation suggest that N₂O reduction is affiliated with a range of physiological responses. Reduction of N₂O is often associated with energy conservation, but that does not appear to be the case for all microorganisms possessing *nosZ*. Instead, it is a way to cope with potentially toxic effects of N₂O or functions as an electron sink for metabolism. Sullivan *et al.* [45] demonstrated that copper limitation downregulates expression of *nosZ* in *Paracoccus denitrificans*, resulting in cytotoxic effects of N₂O due to inactivation of vitamin B₁₂. *Gemmatimonas aurantiaca* strain T-27, which lacks a complete set of denitrification genes and cannot grow under anoxic

Glossary

Chemodenitrification: a chemical reaction between a reduced chemical [e.g., Fe(II) or reduced humic substances] and NO₂[−] to produce NO and N₂O.

Denitrification: an anaerobic respiratory pathway consisting of the sequential reduction of soluble nitrate (NO₃[−]) or nitrite (NO₂[−]) to the gaseous products N₂O and N₂. The pathway can be truncated and terminate with N₂O. A special case is nitrifier-denitrification, in which NH₃ oxidation is coupled to NO₂[−] reduction, which results in the production of N₂O through biotic or a combination of biotic and abiotic reactions.

Fitness: a measure of the reproductive success of a particular genotype during natural selection compared to other genotypes in a given environment.

Genome: an organism's complete set of genetic information, which includes all genes.

Homolog (gene): homologous genes have a common ancestor and share sequence similarities.

Metagenomics: DNA-based analyses where 'all' genetic information in a sample is sequenced. Sequence depth will determine the coverage.

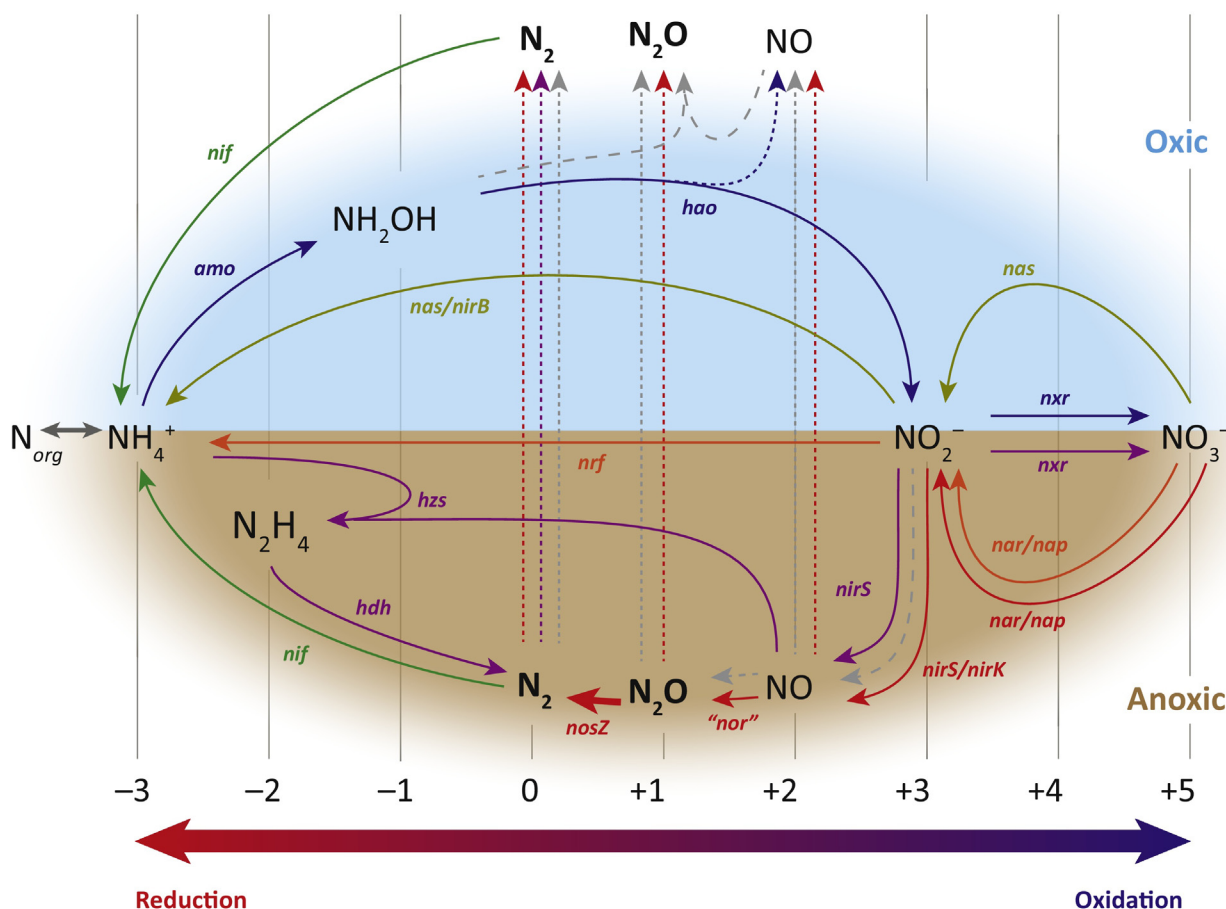
Niche: the ecological role of an organism within a community with respect to use of resources.

Phylogeny: the inferred evolutionary relationship of a set of genes, proteins, genomes based on the similarities and differences in their characteristics.

Trait: an organism's functional characteristic that is a product of genetic composition and environmental factors.

Translocation pathway: proteins that have evolved to function outside the cytoplasmic membrane can be translocated through the membrane by different mechanisms.

Nitrogen fixation
 Nitrate assimilation
 Nitrification
 Denitrification
 Respiratory ammonification (DNRA)
 ANAMMOX
 Chemodenitrification/hybrid N_2O formation



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Figure 1. Microbial Pathways in the Nitrogen (N) Cycle. Background colors differentiate between processes occurring under oxic or anoxic conditions. The compounds are positioned according to the oxidation state of N. Solid colored lines indicate microbial pathways, and the genes encoding the respective enzymes that catalyze each step in the pathway are indicated as follows: *amo*, ammonia monooxygenase; *hao*, hydroxylamine oxidoreductase; *hds* (also known as *hzo*), hydrazine dehydrogenase; *hds*, hydrazine synthase; *nar/nap*, cytoplasmic and periplasmic dissimilatory nitrate reductases, respectively; *nas*, assimilatory nitrate reductase; *nif*, nitrogenase; *nas/nirB*, assimilatory nitrite reductase; *nirS/nirK*, cytochrome-*cd*₁ and copper-based dissimilatory nitrite reductases, respectively; *nor*, any of the multi-heme or heme-copper nitric oxide reductases; *nosZ*, nitrous oxide reductase; *nrf*, formate-dependent dissimilatory nitrite reductase; *nrx*, nitrite oxidoreductase. Dotted lines indicate the contributing pathways to atmospheric loading of gaseous nitrogen compounds, whereas dashed gray lines indicate biotic/abiotic processes generating gaseous N compounds that also feed into the overall N-cycle. DNRA, dissimilatory nitrate reduction to ammonium.

conditions, reduces N_2O following growth under oxic conditions [46]. Here, N_2O apparently acts as an electron sink, and *NosZ* activity sustains viability during temporary anoxia. Interestingly, *nosZ* genes affiliated with the phylum Gemmatimonadetes are among the most frequently encountered *nosZ* genes in many terrestrial ecosystems [47,48].

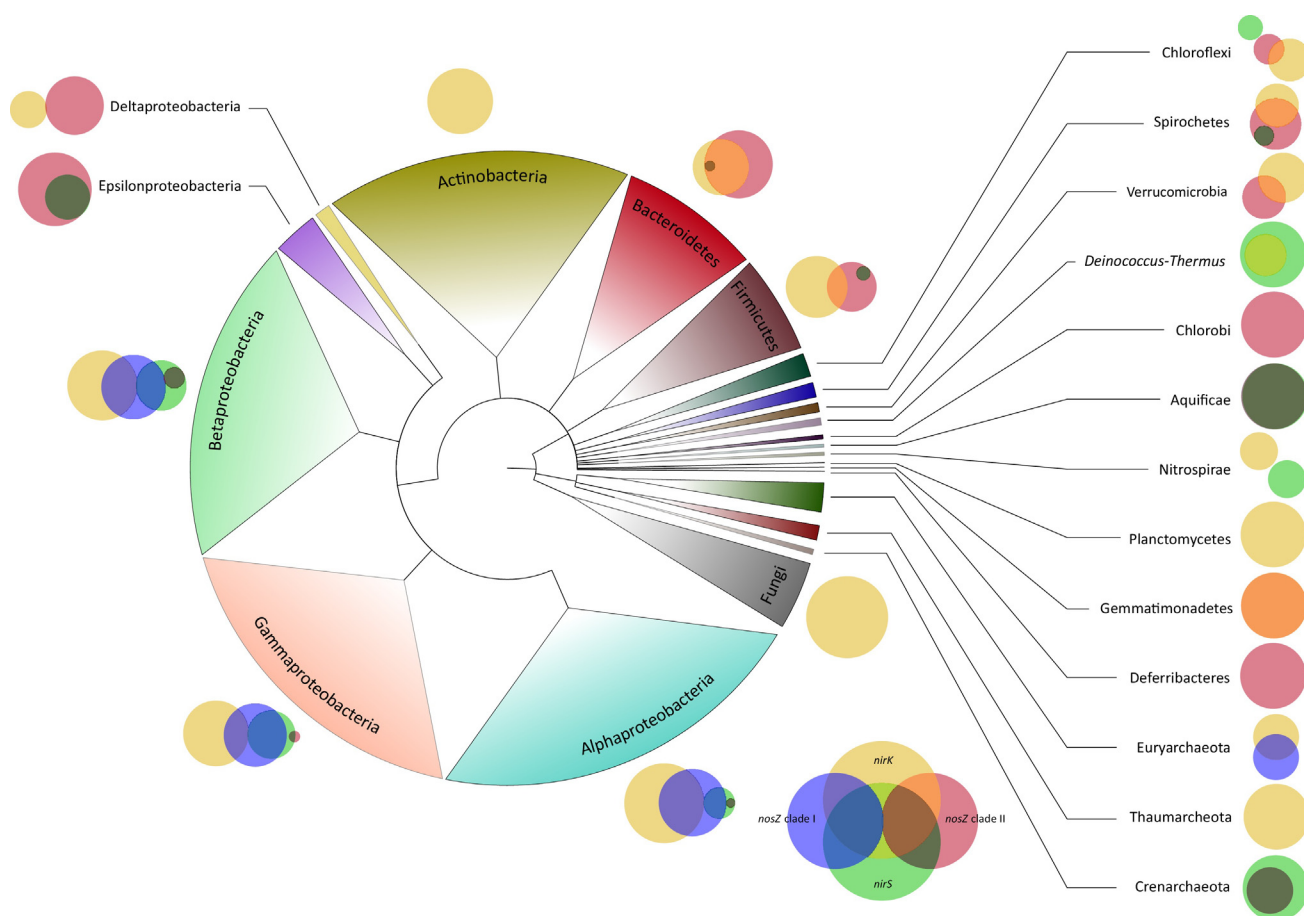
Box 1. Microbial Processes Generating Nitrous Oxide (N₂O)

Nitrous oxide is produced by many different microbial guilds as part of various pathways, in which inorganic nitrogenous compounds are transformed (Figure 1). The relative contribution to net N₂O emissions of the different processes varies considerably and is influenced by the microbial community and environmental condition. However, the majority of N₂O emitted is primarily attributed to denitrification and ammonia oxidation to NO₂[−], the first step in the nitrification process. Denitrification often includes reduction of N₂O to N₂, but denitrifiers may not always perform complete denitrification, resulting in the formation of N₂O as terminal product – either because they lack the genetic capacity for N₂O reduction to N₂ or because environmental factors suppress the reaction [4,19,73]. An example of the former are fungi that denitrify [81–83]. Ammonia oxidation is an energy-yielding, aerobic process in which NH₃ is oxidized to NO₂[−] via the intermediate NH₂OH. While N₂O can be produced from abiotic oxidation of NH₂OH [84], it has been shown that NH₃ oxidation coupled to enzymatic NO₂[−] and NO reduction (i.e., nitrifier-denitrification) is the main route for N₂O emissions during ammonia oxidation [85]. Recent studies show that ammonia-oxidizing archaea generate N₂O through hybrid formation via a spontaneous reaction of NH₂OH and NO, although they likely produce less N₂O than the ammonia-oxidizing bacteria [86–88]. During respiratory ammonification (dissimilatory nitrate reduction to ammonium (DNRA)), NO₃[−]/NO₂[−] is reduced to NH₄⁺ with the concomitant formation of small amounts of N₂O (e.g., [89]). The N₂O is produced when NO in the cell is detoxified [90]. Some DNRA bacteria can also link N₂O reduction to N₂ with energy conservation [91]. The contribution of respiratory ammonification to overall N₂O budgets is uncertain, although most reports conclude that the process contributes marginally to N₂O emissions compared to denitrification and ammonia oxidation [85,92]. Nitrite also reacts chemically with ferrous iron to form NO, which reacts further with ferrous iron to form N₂O, and this chemodenitrification process can make relevant contributions to N₂O formation in environments with active ferric-iron-reducing microorganisms. Nitrous oxide production by methane-oxidizing bacteria, nitrite oxidizing bacteria, and anaerobic methane- and anaerobic ammonium-oxidizing bacteria have also been reported [93–96] but their contributions to global N₂O emissions are unclear.

When N₂O is used for respiration by denitrifiers, reduction may not occur with the same kinetics [18], suggesting adaptations to different environmental conditions. Recent work on the kinetics and regulation of N₂O reduction by *Bacillus vireti* highlights how environmental conditions control the possibility of an organism with clade II *nosZ* acting as a potential N₂O sink [49,50]. Reduction of N₂O was dependent on nitrate concentrations in the culture medium, with higher concentrations (>30 mM) inhibiting NosZ activity. *nosZ* was highly expressed at lower nitrate levels (5 mM), and reduction of exogenous N₂O was coupled to growth, provided that low levels of nitrate or nitrite were present to induce expression. A recent comparison of five bacterial species that sustain growth with N₂O as the only electron acceptor showed that those with clade II *nosZ* exhibited lower whole-cell half-saturation constants (K_s values) than those with clade I *nosZ*, although the latter tended to show higher maximum N₂O reduction rates [20]. The clade II bacteria produced 50–80% more biomass per mol of N₂O reduced, indicating an N₂O respiratory chain that allows for more efficient free energy conservation compared to the clade I system in the studied organisms. Whether this observation can be generalized to all organisms with clade II *nosZ* has yet to be confirmed. Should clade II *nosZ* organisms indeed have different N₂O reduction kinetics from those with clade I, this could explain niche differentiation and would have ecological and environmental consequences.

Ecology of N₂O-Reducing Communities

After the new lineages of N₂O-reducing microorganisms were described, microbial ecologists studied these microorganisms in different environments. A number of studies assessed the diversity and abundance of N₂O reducers to determine factors explaining their distribution. The diversity is typically higher for *nosZ* clade II than for clade I (e.g., [47,51]), which would be expected due to the larger taxonomic breadth of *nosZ* clade II among sequenced genomes [19]. The majority of environmental clade I *nosZ* sequences are most similar to *nosZ* from Alpha- and Betaproteobacteria (Burkholderiales) in both marine and terrestrial habitats [47,51–54]. Within the Alphaproteobacteria, Rhodobacterales dominate in marine sediments and Rhizobiaceae in soils. Clade II *nosZ* sequences are predominantly affiliated with Bacteroidetes, Gemmatimonadetes, and Deltaproteobacteria in most environments, although Gammaproteobacteria and unclassified archaea are also commonly detected in marine and estuary sediments [47,48,51–56]. Several environmental clade II *nosZ* reads are assigned to

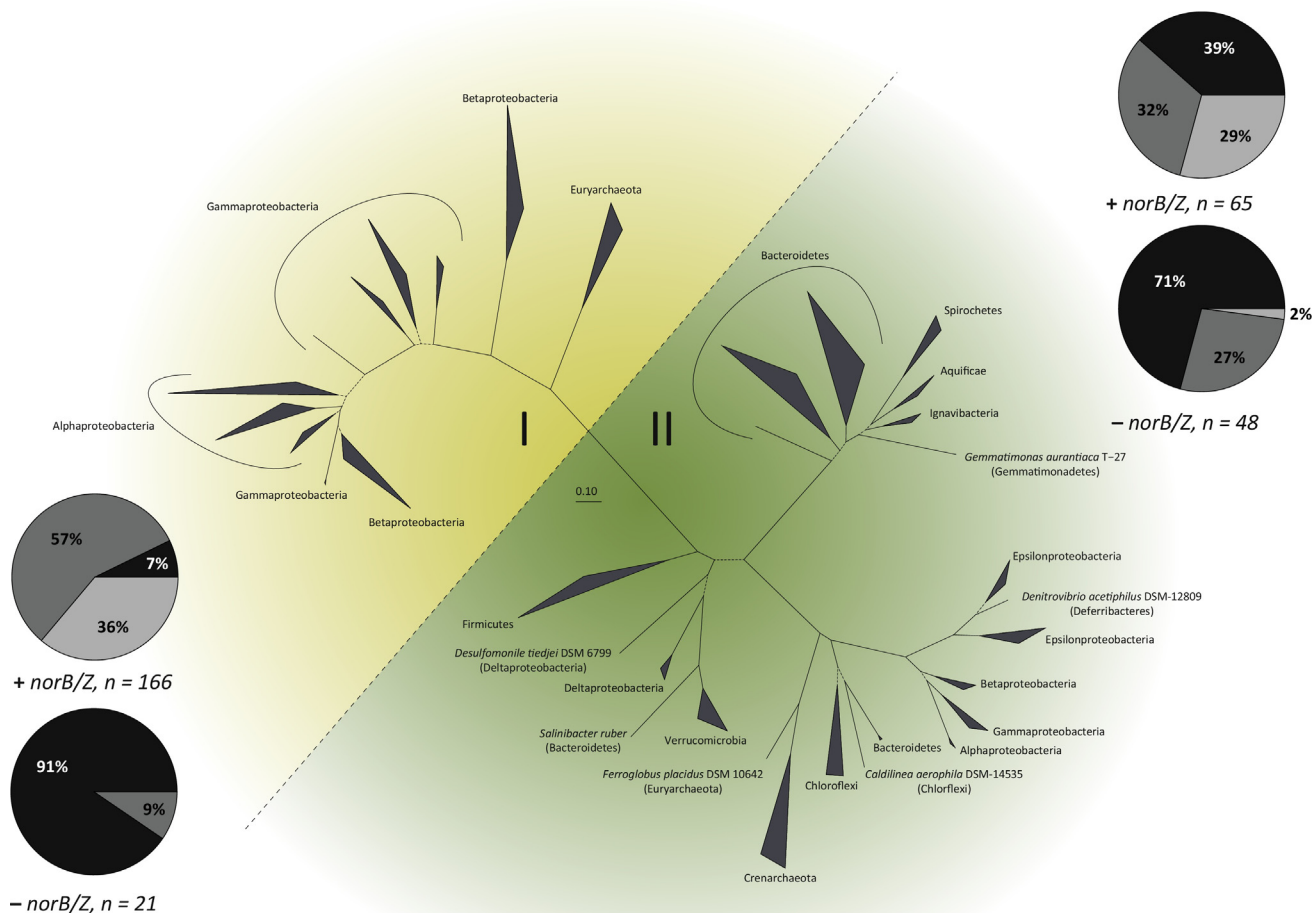


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Figure 2. Taxonomic Distribution of Denitrifier and N₂O Reducer Genomes. Genomes harboring genes encoding the copper- and cytochrome *d*₁-type nitrite reductases (*nirK* and *nirS*, respectively) involved in denitrification, as well as the different variants of the *nosZ* gene encoding the N₂O reductase. The center tree is based on NCBI taxonomic rankings, with clade size proportional to the number of genomes found within each phylum or, for the Proteobacteria, class grouping. Venn diagrams illustrate the frequency of *nir* and *nosZ* gene co-occurrences in the genomes of each taxonomic group, where circle size is proportional to the frequency of gene occurrence within each group, and overlap indicates degree of gene co-occurrences. Color-coding for each combination is shown at lower right. Based on data reported in [19].

nondenitrifying genomes of genera such as *Anaeromyxobacter*, *Dyadobacter*, *Gemmatimonas*, *Ignavibacterium*, *Melioribacter*, and *Pedobacter*. These organisms could be using N₂O originating from other organisms, chemodenitrification, or other sources [13,55,57] (Box 2). Their widespread distribution indicates that the ability to reduce N₂O is an ecologically relevant trait.

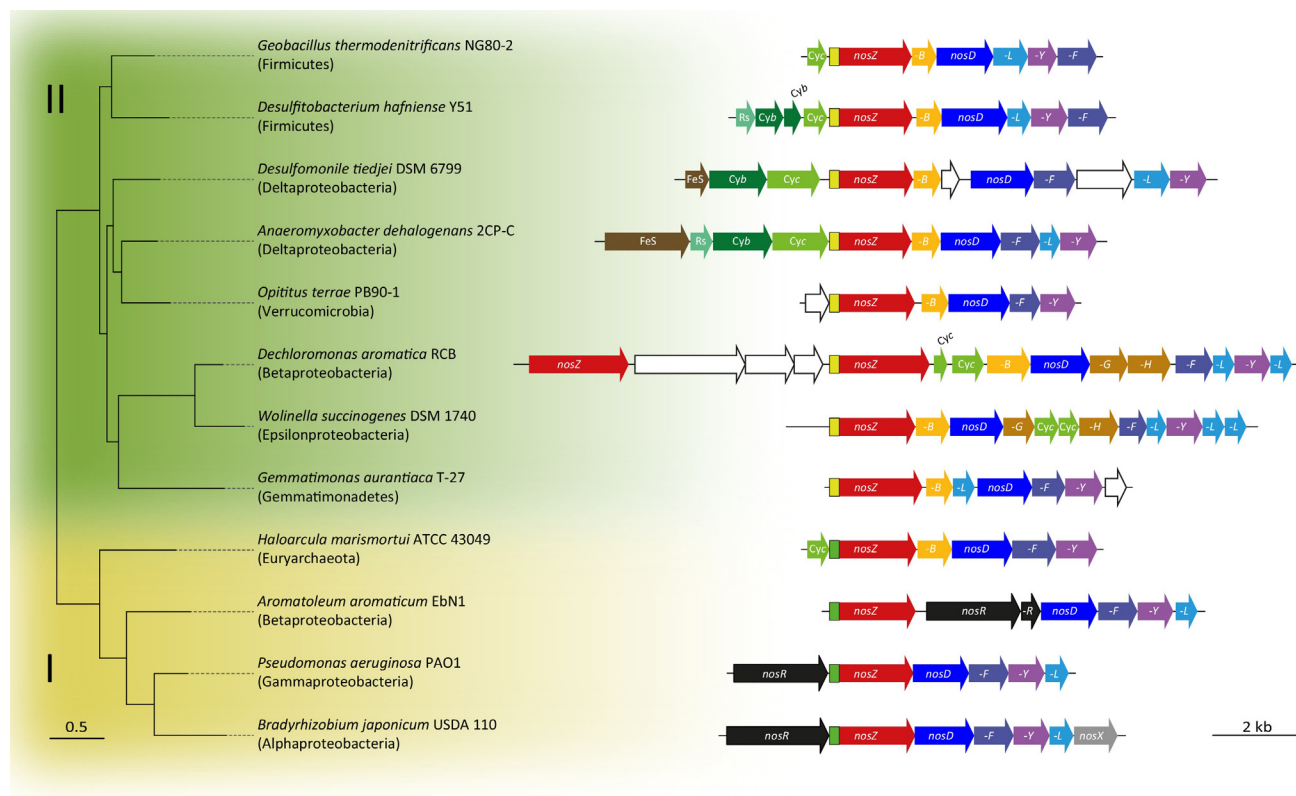
Pioneering work showed that clade II *nosZ* was equally or more abundant than clade I in a range of environments, for example, soils, wastewater-treatment plants, wetlands, and rice paddies [14]. Subsequent studies have mainly focused on terrestrial environments, especially agricultural soils due to their large contribution to N₂O emissions. Numerical dominance of clade II *nosZ* over clade I, with ratios of 1.5–10, is commonly reported when using quantitative PCR [14,58,59], although there are soils with both higher and lower ratios [47,52,56]. Studies using **metagenomics**, which circumvents primer bias, supports the dominance of clade II *nosZ* in several terrestrial habitats [48,54]. Nitrous oxide reducers in aquatic and engineered systems, for example, wastewater-treatment plants, have so far received less attention. In a salt marsh,



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Figure 3. Maximum-Likelihood-Based Phylogeny of NosZ Amino-Acid Sequences Showing the Two Major Clades of NosZ (I and II). Major lineages of monophyletic taxonomic composition at the class or phylum level are collapsed, with polygon size proportional to amino-acid sequence diversity within each grouping, and dotted branches indicating bootstrap support less than 70%. Pie charts illustrate the percentage of genomes with each *nosZ* variant that lacks the *nir* genes associated with denitrification (black), or possesses either *nirS* (light grey) or *nirK* (dark grey). Percentages are shown for genomes with and without the *norB/Z* genes that encode the heme-copper nitric oxide reductase that reduces NO to N₂O. The number of genomes for each group is shown below each pie chart. Based on data reported in [19].

clade II *nosZ* was three times more abundant than clade I *nosZ* based on metagenomics analysis [53]. In PCR-based studies of aquatic environments, the two clades were either equally abundant or clade I *nosZ* dominated. In wetlands, boreal lakes, and a eutrophic lagoon, clade I *nosZ* was only slightly more abundant than clade II *nosZ* [60–62], but up to 1000 times more abundant in constructed wetlands and in a bioreactor treating nitrate-rich water [63,64]. Also in coastal sediments and salt marshes, clade I *nosZ* occurred at a ≥ 10 -fold higher abundance than clade II *nosZ* [51,65]. Based on the abundance patterns, complete denitrifiers, being overrepresented in clade I *nosZ* [19], could have an important role in N₂O reduction in aquatic systems, although activity needs to be confirmed. By contrast, organisms with clade II *nosZ* would, in general, be more relevant in soils. The high abundance of clade II *nosZ* microorganisms, together with their great diversity in the environment, emphasizes N₂O reduction as an ecologically favorable trait, which potentially controls net N₂O emissions from ecosystems.



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Figure 4. Comparison of *nos* Gene Clusters from Genomes Harboring Clade I or Clade II *nosZ* Genes. Phylogeny at right is based on the phylogeny shown in Figure 2, with phylum or class level taxonomic designation under each name, and the scale bar indicating estimated amino-acid substitutions per site. For each gene cluster, *nosZ* and its accessory genes (*BDFGHLRXY*) are labeled and colored according to homology across the different gene clusters. For each *nosZ* reading frame, N-terminal regions are colored according to signal peptides targeting the Sec (yellow) or twin-arginine translocation (TAT; green) pathways that insert the *nosZ* product into the periplasmic space. Additional proteins, including iron-sulfur-binding proteins (FeS), Rieske iron-sulfur proteins (S), and *b*- and *c*-type cytochromes (Cyb and Cyc, respectively) are also labeled. Noncolored genes denote open reading frames with no orthologs in other *nos* gene clusters, and the scale bar at lower-right indicates gene size. Adapted from [13].

Contrasting patterns in terms of abundance and diversity of the two main *nosZ* clades were typically observed within the studies referenced above, which suggest niche differentiation between the two clades. Nevertheless, there are few specific factors that correlate with one or the other clade when comparing across individual studies. Soil textural components, for example, clay or loam content [47,58,62], as well as nutrient status, for example, nitrogen, carbon, organic matter, or C:N ratio [47,53,58,61,65], were shown to promote the size of the *nosZ* clade I community, yet no specific factors favoring *nosZ* clade II have emerged. Thus, underlying drivers are only indirectly captured or context-dependent, and biotic drivers or interactions could have been overlooked. Interestingly, microorganisms harboring either gene occupied different ecological niches in the root-soil interface, with *nosZ* clade II types favored in the soil compartment and type I associated with crop roots regardless of soil type [19]. In agreement, the abundance ratio of clade I and clade II *nosZ* was higher in the rhizosphere of silver birch compared to bulk soil [66]. Both of these studies indicate a tighter link between N_2O production and reduction via the denitrification pathway in the rhizosphere compared to bulk soil (Box 2). Given the phylogenetic diversity of organisms with the capacity to reduce N_2O , it is not surprising that different niches exist also within each of the two clades. For example, niche partitioning for both clade I and clade II *nosZ* was evidenced in relation to oxygen availability,

Box 2. Hypothesized Interactions between N_2O Producers and N_2O Reducers

Since N_2O is formed in a number of processes, but only consumed via the reduction to N_2 , interactions between the producers and reducers can be assumed. Indeed, consumption of N_2O was demonstrated experimentally in soil microcosms inoculated with a nondenitrifying bacterium capable of eliminating N_2O produced by the native microbial community [57]. The denitrification pathway could potentially also be split over multiple taxa as the pathway is modular [8,19], consisting of a suite of functions that can either act in concert or be performed individually, depending on genomic or environmental constraints. This raises several questions. Is this modularity an example of labor division in communities assembled into consortia to carry out a cooperative function? If so, why is the pathway truncated for some microorganisms but not for others? In other words, what are the fitness advantages of complete and truncated denitrification pathways, and under which conditions are they favored? It can be hypothesized that substrate cross-feeding eliminates interenzyme competition and accelerates substrate consumption. This was demonstrated in experiments with isogenic mutants of the complete denitrifier *Pseudomonas stutzeri*, as nitrate and nitrite reductases, when functional in separate populations in the same culture, no longer competed for electron donors as they would if functioning in the same cell, thereby avoiding accumulation of growth-inhibiting levels of nitrite [97]. Cross-feeding of N_2O among complete or partial denitrifiers and N_2O reducers may also be advantageous, as suggested by the substantial number of genomes with *nosZ* that do not possess a denitrification pathway. This type of interaction was inferred after genomic reconstruction of shotgun sequence reads from an estuary showing that genes for a truncated denitrification pathway – terminating in N_2O – were present in populations of Gammaproteobacteria, while the ability to reduce N_2O was found only in Gemmatimonadetes and Myxococcales that possess clade II *nosZ* [55]. Another possible role for *NosZ* in nondenitrifiers would be to scavenge N_2O generated by other biotic as well as abiotic processes (see Box 1 and Glossary). A recent survey using stable N isotopes revealed that chemodenitrification drove a significant fraction of the N_2O generation in estuarine sediments [26]. Since *Anaeromyxobacter dehalogenans* 2CP-C constitutively expresses Fe(III)-reducing activity and can reduce NO_3^- to NO_2^- and N_2O to N_2 [98,99], one could hypothesize that this bacterium reduces N_2O generated via chemodenitrification. This would be an example of a nondenitrifier that competes with denitrifiers by coupling biotic and abiotic reactions.

eutrophication level, pH, C:N ratios, cropping systems, and biochar addition to agricultural soil [51–53,56,67].

Ramifications for Ecosystem Functioning

The diversity of the N_2O -producing and N_2O -reducing communities ultimately determines the net N_2O emissions, since communities with different genetic potentials (e.g., assemblages of various functional modules) are subject to ecological processes that influence their abundance, diversity, and activity. The feedback between dynamic environmental conditions and evolutionary processes will determine the structure and diversity of N_2O -producing and N_2O -reducing communities. By ‘structure and diversity’, we mean not only the assemblage of taxa that constitute the communities, but also the association of the various processes within the denitrification pathway or in association with other processes. Early work suggested that differences in relative rates of N_2O production between an agricultural and a successional field could only be explained by the denitrifier community composition when controlling all known environmental factors regulating N_2O production and consumption by denitrification [68]. However, the influence of the denitrifier community composition and the modularity of the denitrification pathway are interwoven since co-occurrence patterns of denitrification genes are not randomly distributed across taxa [19]. For instance, the higher frequency of co-occurrence of *nosZ* with *nirS* than with *nirK* (Figure 3) suggests that *nirS* type denitrifiers are more likely to perform complete denitrification, and thereby contribute less to N_2O emissions, than is the case for *nirK* type denitrifiers. A field study reported a positive correlation between *nirK* abundance and N_2O emissions, and qPCR analyses suggested that only 10–30% of the *nirK* type denitrifiers also carried the *nosZ* gene [69]. The putative importance of a truncation in the last step of the denitrification pathway was corroborated by a negative relationship between the proportion of bacteria with *nosZ* and the N_2O -to- N_2 denitrification end-product ratio in a pasture [70]. Similarly, interlake variation in N_2O accumulation was connected to the relative abundance of *nir* versus *nosZ* genes [60]. Other examples of such correlations have been reported in different ecosystems, for example [71,72], but experimental testing of causal relationships is needed. Addition of *Agrobacterium tumefaciens* C58, a denitrifying strain lacking *nosZ* and therefore terminating with N_2O , to different soils resulted in an increase in

the relative amount of N_2O emitted [73]. This direct manipulation experiment provides a proof of principle that the inability to synthesize NosZ can influence the ratio of the denitrification end-products, and that the extent of the reduction of N_2O to N_2 has both genetic and environmental controls.

After the finding that *nosZ* could be delineated into two distinct clades, one of the questions raised was whether differences between microorganisms with clade I and II *nosZ* could impact net N_2O emissions. In earlier studies considering only the clade I *nosZ* community, the link between *nosZ* community metrics and N_2O emissions ranges from being strong, weak, or even absent [74–77]. However, a recent landmark paper highlighted the putative role of the clade II *nosZ* rather than clade I *nosZ* community for the capacity of soils to act as N_2O sinks [47]. This was supported by later studies linking the clade II *nosZ* community with reduced potential N_2O emissions [52,54]. These observed differential roles are consistent with the finding that about half of clade II *nosZ* microorganisms lack *nir* genes while most of the clade I *nosZ* types possess *nirK* or *nirS* [19]. The proposed role of clade II *nosZ* microorganisms in the soil N_2O -reduction capacity was recently verified [57]. Inoculation of *Dyadobacter fermentans* – a nondenitrifying bacterium possessing clade II *nosZ* and commonly detected in soil metagenomes [48] – to different soils resulted in a decrease in N_2O emission of up to 189% in one-third of the inoculated soils [57]. This demonstrates that clade II *nosZ* bacteria can consume N_2O emitted in soil by other microorganisms, and suggests that a tighter link between N_2O producers and reducers could have relevance for mitigating N_2O emissions (Box 2). It has been known anecdotally for some years that soils can consume N_2O *in situ* [78–80], but the mechanisms have not been investigated. Further work is needed to determine if N_2O emissions are controlled by N_2O production or N_2O consumption, why and when soils increase N_2O consumption, and whether microbial N_2O reduction can be important, in quantitative terms, for curbing global N_2O emissions.

Concluding Remarks

The recent discovery of clade II *nosZ*, and the realization that nondenitrifying N_2O -reducing microorganisms are present in high numbers and great diversity in essentially all ecosystems, has opened up a new field of research. Research conducted over the past 5 years has substantially expanded our understanding of the organismal, genomic, and genetic diversity of the functional units that drive N_2O reduction to N_2 and underscores the assertion made by Zumft and Kroneck [40], that N_2O reduction ‘represents a respiratory process in its own right’. Both N_2O production and N_2O reduction are integral parts of the nitrogen cycle, yet most ecosystems emit N_2O . Emission of N_2O is of environmental concern, and experiments and surveys comparing N_2O reducers within clade I and clade II suggest that clade II organisms are key determinants of a soil’s N_2O sink capacity. More insight into the ecology of the N_2O reducers is needed, especially mechanisms and factors explaining niche differentiation between organisms with clade I and II *nosZ*, and between organisms within each of the clades. Additional questions to further our understanding revolve around what the main purpose of N_2O reduction is in the environment, whether close interactions between N_2O producers and N_2O reducers are common, and if there are advantages of labor division in consortia splitting the denitrification pathway (see Outstanding Questions). This review highlights advancements in the field, but further progress is essential for developing predictive understanding of N_2O fluxes and generating improved management strategies that can curb N_2O emissions.

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Outstanding Questions

What is the main purpose of reduction of N_2O in the environment? Microorganisms can reduce N_2O for different purposes, that is, energy conservation, detoxification, or removal of excess electrons. Environmental sequences indicate that such functional variation is present, if we can assume that sequence similarity equals functional similarity.

Are responses to environmental conditions conserved within different lineages in the N_2O reductase phylogeny? Similarly, are efficiencies of N_2O reduction, when coupled with energy conservation, conserved in the phylogeny? This type of information is needed to understand if and how evolution and niche differentiation among and within the two major clades of N_2O reducing microorganisms are linked.

Is niche differentiation between clade I and II organisms mainly due to differences related to the N_2O reductase or are other traits that vary between these two groups more important in this context. The distribution of the two types of N_2O reductase is correlated to taxa, and thereby interlinked. This needs to be teased apart to be able to manipulate and manage the N_2O reducers in managed ecosystems.

Are direct associations between N_2O producers and N_2O reducers common, and if so, what are the advantages for the organisms?

Do the rates of N_2O formation or the rates of N_2O reduction control N_2O emission, and is it ecosystem-dependent? Knowledge of underlying emission controls could aid in developing and improving strategies to decrease N_2O emissions.

References

- IPCC (2013) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University Press
- Ravishankara, A.R. *et al.* (2009) Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. *Science* 326, 123–125
- Conrad, R. (1996) Soil microorganisms as controllers of atmospheric trace gases (H_2 , CO, CH_4 , OCS, N_2O and NO). *Microbiol. Rev.* 60, 609–640
- Firestone, M.K. *et al.* (1980) Nitrous-oxide from soil denitrification – factors controlling its biological production. *Science* 208, 749–751
- Seitzinger, S. *et al.* (2000) Global distribution of N_2O emissions from aquatic systems: natural emissions and anthropogenic effects. *Chemosphere* 2, 267–279
- Bouwman, L. *et al.* (2013) Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900–2050 period. *Proc. Natl. Acad. Sci. U. S. A.* 110, 20882–20887
- Smith, K.A. *et al.* (2012) The role of N_2O derived from crop-based biofuels, and from agriculture in general, in Earth's climate. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 367, 1169–1174
- Zumft, W.G. (1997) Cell biology and molecular basis of denitrification. *Microbiol. Mol. Biol. Rev.* 61, 533–616
- Philippot, L. (2002) Denitrifying genes in bacterial and Archaeal genomes. *Biochim. Biophys. Acta* 1577, 355–376
- Payne, W.J. *et al.* (1982) Nitrogen-oxide reduction in *Wolinella succinogenes* and *Campylobacter* species. *J. Bacteriol.* 152, 915–918
- Simon, J. *et al.* (2004) The unprecedented *nos* gene cluster of *Wolinella succinogenes* encodes a novel respiratory electron transfer pathway to cytochrome c nitrous oxide reductase. *FEBS Lett.* 569, 7–12
- Jones, C.M. *et al.* (2011) Phenotypic and genotypic heterogeneity among closely related soil-borne N_2 - and N_2O -producing *Bacillus* isolates harboring the *nosZ* gene. *FEMS Microbiol. Ecol.* 76, 541–552
- Sanford, R.A. *et al.* (2012) Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils. *Proc. Natl. Acad. Sci. U. S. A.* 109, 19709–19714
- Jones, C.M. *et al.* (2013) The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. *ISME J.* 7, 417–426
- Richardson, D. *et al.* (2009) Mitigating release of the potent greenhouse gas N_2O from the nitrogen cycle – could enzymic regulation hold the key? *Trends Biotechnol.* 27, 388–397
- Thomson, A.J. *et al.* (2012) Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 367, 1157–1168
- Hu, H.-W. *et al.* (2015) Microbial regulation of terrestrial nitrous oxide formation: understanding the biological pathways for prediction of emission rates. *FEMS Microbiol. Rev.* 39, 729–749
- Cavigelli, M. and Robertson, G. (2001) Role of denitrifier diversity in rates of nitrous oxide consumption in a terrestrial ecosystem. *Soil Biol. Biochem.* 33, 297–310
- Graf, D.R.H. *et al.* (2014) Intergenomic comparisons highlight modularity of the denitrification pathway and underpin the importance of community structure for N_2O emissions. *PLoS One* 9, e114118
- Yoon, S. *et al.* (2016) Nitrous oxide reduction kinetics distinguish bacteria harboring Clade I *NosZ* from those harboring Clade II *NosZ*. *Appl. Environ. Microbiol.* 82, 3793–3800
- Jones, C.M. *et al.* (2008) Phylogenetic analysis of nitrite, nitric oxide, and nitrous oxide respiratory enzymes reveal a complex evolutionary history for denitrification. *Mol. Biol. Evol.* 25, 1955–1966
- Zerkle, A.L. *et al.* (2017) Onset of the aerobic nitrogen cycle during the Great Oxidation Event. *Nature* 542, 465–467
- Fru, E.C. *et al.* (2016) Cu isotopes in marine black shales record the Great Oxidation Event. *Proc. Natl. Acad. Sci. U. S. A.* 113, 4941–4946
- Canfield, D. *et al.* (2006) Early anaerobic metabolisms. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 361, 1819
- Coby, A.J. and Picardal, F.W. (2005) Inhibition of NO_3^- and NO_2^- reduction by microbial Fe(III) reduction: evidence of a reaction between NO_2^- and cell surface-bound Fe_2^+ . *Appl. Environ. Microbiol.* 71, 5267–5274
- Buchwald, C. *et al.* (2016) Constraining the role of iron in environmental nitrogen transformations: Dual stable isotope systematics of abiotic NO_2^- reduction by Fe(II) and its production of N_2O . *Geochim. Cosmochim. Acta* 186, 1–12
- Barth, K.R. *et al.* (2009) Biochemical and genomic analysis of the denitrification pathway within the genus *Neisseria*. *Microbiology* 155, 4093–4103
- Liu, B. *et al.* (2013) Strains in the genus *Thauera* exhibit remarkably different denitrification regulatory phenotypes. *Environ. Microbiol.* 15, 2816–2828
- Verbaendert, I. *et al.* (2014) Primers for overlooked *nirK*, *qnorB*, and *nosZ* genes of thermophilic Gram-positive denitrifiers. *FEMS Microbiol. Ecol.* 89, 162–180
- Nie, Y. *et al.* (2016) Physiological and genotypic characteristics of nitrous oxide (N_2O)-emitting pseudomonas species isolated from dent corn and soil farmland in Hokkaido, Japan. *Microbes Environ.* 31, 93–103
- Vial, L. *et al.* (2006) Phase variation and genomic architecture changes in *Azospirillum*. *J. Bacteriol.* 188, 5364–5373
- Koskineniemi, S. *et al.* (2012) Selection-driven gene loss in bacteria. *PLoS Genet.* 8, e1002787
- Felgate, H. *et al.* (2012) The impact of copper, nitrate and carbon status on the emission of nitrous oxide by two species of bacteria with biochemically distinct denitrification pathways. *Environ. Microbiol.* 14, 1788–1800
- Kielak, A.M. *et al.* (2016) The ecology of Acidobacteria: Moving beyond genes and genomes. *Front. Microbiol.* 7, 744
- Fierer, N. *et al.* (2007) Toward an ecological classification of soil bacteria. *Ecology* 88, 1354–1364
- Strickland, M.S. *et al.* (2009) Litter quality is in the eye of the beholder: initial decomposition rates as a function of inoculum characteristics. *Funct. Ecol.* 23, 627–636
- Philippot, L. *et al.* (2010) The ecological coherence of high bacterial taxonomic ranks. *Nat. Rev. Microbiol.* 8, 523–529
- Cederlund, H. *et al.* (2014) Soil carbon quality and nitrogen fertilization structure bacterial communities with predictable responses of major bacterial phyla. *Appl. Soil Ecol.* 84, 62–68
- Berks, B. *et al.* (2000) The Tat protein export pathway. *Mol. Microbiol.* 35, 260–274
- Zumft, W. and Kroneck, P. (2007) Respiratory transformation of nitrous oxide (N_2O) to dinitrogen by Bacteria and Archaea. *Adv. Microb. Physiol.* 53, 107–227
- Yoon, S. *et al.* (2015) Denitrification versus respiratory ammonification: environmental controls of two competing dissimilatory NO_3^-/NO_2^- reduction pathways in *Shewanella loihica* strain PV-4. *ISME J.* 9, 1093–1104
- Pohlschröder, M. *et al.* (2005) Diversity and evolution of protein translocation. *Annu. Rev. Microbiol.* 59, 91–111
- Suharti and de Vries, S. (2005) Membrane-bound denitrification in the Gram-positive bacterium *Bacillus azotoformans*. *Biochem. Soc. Trans.* 33, 130–133
- van Spanning, R. (2011) Structure, function, regulation and evolution of the nitrite and nitrous oxide reductases: denitrification enzymes with a beta-propeller fold. In *Nitrogen Cycling in Bacteria: Molecular Analysis* (Moir, J.W.B., ed.), pp. 135–161, Caister Academic Press
- Sullivan, M.J. *et al.* (2013) Copper control of bacterial nitrous oxide emission and its impact on vitamin B₁₂-dependent metabolism. *Proc. Natl. Acad. Sci. U. S. A.* 110, 19926–19931

46. Park, D. *et al.* (2017) Nitrous oxide reduction by an obligate aerobic bacterium *Gemmatimonas aurantiaca* strain T-27. *Appl. Environ. Microbiol.* 83, e00502–17
47. Jones, C.M. *et al.* (2014) Recently identified microbial guild mediates soil N₂O sink capacity. *Nat. Clim. Change* 4, 801–805
48. Orellana, L. *et al.* (2014) Detecting nitrous oxide reductase (*nosZ*) genes in soil metagenomes: method development and implications for the nitrogen cycle. *mBio* 5, e01193–14
49. Mania, D. *et al.* (2014) The nitrate-ammonifying and *nosZ*-carrying bacterium *Bacillus vireti* is a potent source and sink for nitric and nitrous oxide under high nitrate conditions. *Environ. Microbiol.* 16, 3196–3210
50. Mania, D. *et al.* (2016) Regulation of nitrogen metabolism in the nitrate-ammonifying soil bacterium *Bacillus vireti* and evidence for its ability to grow using N₂O as electron acceptor. *Environ. Microbiol.* 18, 2937–2950
51. Wittorf, L. *et al.* (2016) Habitat partitioning of marine benthic denitrifier communities in response to oxygen availability. *Environ. Microbiol. Rep.* 8, 486–492
52. Domeignoz-Horta, L. *et al.* (2015) The diversity of the N₂O reducers matters for the N₂O:N₂ denitrification end-product ratio across an annual and a perennial cropping system. *Front. Microbiol.* 6, 971
53. Graves, C. *et al.* (2016) Functional responses of salt marsh microbial communities to long-term nutrient enrichment. *Appl. Environ. Microbiol.* 82, 2862–2871
54. Samad, M. *et al.* (2016) Phylogenetic and functional potential links pH and N₂O emissions in pasture soils. *Sci. Rep.* 6, 35990
55. Baker, B. *et al.* (2015) Genomic resolution of linkages in carbon, nitrogen, and sulfur cycling among widespread estuary sediment bacteria. *Microbiome* 3, 14
56. Harter, J. *et al.* (2016) Soil biochar amendment shapes the composition of N₂O-reducing microbial communities. *Sci. Total Environ.* 562, 379–390
57. Domeignoz-Horta, L. *et al.* (2016) Non-denitrifying nitrous oxide-reducing bacteria – an effective N₂O sink in soil. *Soil Biol. Biochem.* 103, 376–379
58. Tsiknia, M. *et al.* (2015) Environmental drivers of the distribution of nitrogen functional genes at a watershed scale. *FEMS Microbiol. Ecol.* 91, fiv052
59. Graf, D.R.H. *et al.* (2016) Soil type overrides plant effect on genetic and enzymatic N₂O production potential in arable soils. *Soil Biol. Biochem.* 100, 125–128
60. Saareheimo, J. *et al.* (2015) Genetic and environmental controls on nitrous oxide accumulation in lakes. *PLoS One* 10, e0121201
61. Ligi, T. *et al.* (2015) The genetic potential of N₂ emission via denitrification and ANAMMOX from the soils and sediments of a created riverine treatment wetland complex. *Ecol. Eng.* 80, 181–190
62. Highton, M. *et al.* (2016) Physical factors correlate to microbial community structure and nitrogen cycling gene abundance in a nitrate fed eutrophic lagoon. *Front. Microbiol.* 7, 1691
63. Herbert, R. *et al.* (2014) Nitrogen removal and spatial distribution of denitrifier and anammox communities in a bioreactor for mine drainage treatment. *Water Res.* 66, 350–360
64. Paranychanakis, N. *et al.* (2016) Pathways regulating the removal of nitrogen in planted and unplanted subsurface flow constructed wetlands. *Water Res.* 102, 321–329
65. Dini-Andreote, F. *et al.* (2016) Reconstructing the genetic potential of the microbially-mediated nitrogen cycle in a salt marsh ecosystem. *Front. Microbiol.* 7, 902
66. Truu, M. *et al.* (2017) Elevated air humidity changes soil bacterial community structure in the silver birch stand. *Front. Microbiol.* 8, 557
67. Sun, X. *et al.* (2017) Community composition of nitrous oxide consuming bacteria in the oxygen minimum zone of the Eastern Tropical South Pacific. *Front. Microbiol.* 8, 1183
68. Cavigelli, M. and Robertson, G. (2000) The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology* 81, 1402–1414
69. Clark, I. *et al.* (2012) Impact of nitrogen application rates on the activity and diversity of denitrifying bacteria in the Broadbalk Wheat. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 367, 1235–1244
70. Philippot, L. *et al.* (2009) Mapping field-scale spatial patterns of size and activity of the denitrifier community. *Environ. Microbiol.* 11, 1518–1526
71. Morales, S. *et al.* (2010) Bacterial gene abundances as indicators of greenhouse gas emission in soils. *ISME J.* 4, 799–808
72. Cuhel, J. *et al.* (2010) Insights into the effect of soil pH on N₂O and N₂ emissions and denitrifier community size and activity. *Appl. Environ. Microbiol.* 76, 1870–1878
73. Philippot, L. *et al.* (2011) Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N₂O emissions from soil. *Glob. Change Biol.* 17, 1497–1504
74. Miller, M. *et al.* (2008) Crop residue influence on denitrification, N₂O emissions and denitrifier community abundance in soil. *Soil Biol. Biochem.* 40, 2553–2562
75. Cantarel, A. *et al.* (2012) Four years of experimental climate change modifies the microbial drivers of N₂O fluxes in an upland grassland ecosystem. *Glob. Change Biol.* 12, 2520–2531
76. Braker, G. *et al.* (2012) Genetic characterization of denitrifier communities with contrasting intrinsic functional traits. *FEMS Microbiol. Ecol.* 79, 542–554
77. Cui, P. *et al.* (2016) Long-term organic and inorganic fertilization alters temperature sensitivity of potential N₂O emissions and associated microbes. *Soil Biol. Biochem.* 93, 131–141
78. Chapuis-Lardy, L. *et al.* (2007) Soils, a sink for N₂O? A review. *Glob. Change Biol.* 13, 1–17
79. Wu, D. *et al.* (2013) N₂O consumption by low-nitrogen soil and its regulation by water and oxygen. *Soil Biol. Biochem.* 60, 165–172
80. Ye, R. and Horwath, W.R. (2016) Nitrous oxide uptake in rewetted wetlands with contrasting soil organic carbon contents. *Soil Biol. Biochem.* 100, 110–117
81. Shoun, H. *et al.* (2012) Fungal denitrification and nitric oxide reductase cytochrome P450_{nor}. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 367, 1186–1194
82. Maeda, K. *et al.* (2015) N₂O production, a widespread trait in fungi. *Sci. Rep.* 5, 9697
83. Higgins, S. *et al.* (2016) Detection and diversity of fungal cytochrome nitric oxide reductase (*p450_{nor}*) in two distinct agricultural soils and implications for the nitrogen cycle. *Appl. Environ. Microbiol.* 82, 2919–2928
84. Heil, J. *et al.* (2016) A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil. *Eur. J. Soil Sci.* 67, 23–39
85. Kool, D.M. *et al.* (2011) Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil. *Soil Biol. Biochem.* 43, 174–178
86. Stieglmeier, M. *et al.* (2014) Aerobic nitrous oxide production through N-nitrosating hybrid formation in ammonia-oxidizing archaea. *ISME J.* 8, 1135–1146
87. Hink, L. *et al.* (2016) Archaea produce lower yields of N₂O than bacteria during aerobic ammonia oxidation in soil. *Environ. Microbiol.* Published online April 28, 2016. <http://dx.doi.org/10.1111/1462-2920.13282>
88. Kozłowski, J.A. *et al.* (2016) Pathways and key intermediates required for obligate aerobic ammonia-dependent chemolithotrophy in bacteria and Thaumarchaeota. *ISME J.* 10, 1836–1845
89. Streminska, M.A. *et al.* (2012) Nitrous oxide production in soil isolates of nitrate-ammonifying bacteria. *Environ. Microbiol. Rep.* 4, 66–71
90. Spiro, S. (2012) Nitrous oxide production and consumption: regulation of gene expression by gas-sensitive transcription factors. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 367, 1213–1225
91. Simon, J. and Klotz, M.G. (2013) Diversity and evolution of bioenergetic systems involved in microbial nitrogen compound transformations. *Biochim. Biophys. Acta* 1827, 114–135
92. Rütting, T. *et al.* (2011) Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. *Biogeosciences* 8, 1779–1791

93. Campbell, M.A. *et al.* (2011) Model of the molecular basis for hydroxylamine oxidation and nitrous oxide production in methanotrophic bacteria. *FEMS Microbiol. Lett.* 322, 82–89
94. Stein, L.Y. and Klotz, M.G. (2011) Nitrifying and denitrifying pathways of methanotrophic bacteria. *Biochem. Soc. Trans.* 39, 1826–1831
95. Schreiber, F. *et al.* (2012) Nitric oxide and nitrous oxide turnover in natural and engineered microbial communities: biological pathways, chemical reactions, and novel technologies. *Front. Microbiol.* 3, 372
96. Butterbach-Bahl, K. *et al.* (2013) Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 368, 20130122
97. Lijja, E.E. and Johnson, D.R. (2016) Segregating metabolic processes into different microbial cells accelerates the consumption of inhibitory substrates. *ISME J.* 10, 1568–1578
98. Sanford, R. *et al.* (2002) Characterization and description of *Anaeromyxobacter dehalogenans* gen. nov., sp nov., an arylhalorespiring facultative anaerobic myxobacterium. *Appl. Environ. Microbiol.* 68, 893–900
99. He, Q. and Sanford, R.A. (2003) Characterization of Fe(III) reduction by chlororespiring *Anaeromyxobacter dehalogenans*. *Appl. Environ. Microbiol.* 69, 2712–2718